NON-VIRAL CAUSES OF DIARRHOEA IN CHILDREN LESS THAN 5 YEARS FROM SENTINEL SITES IN SOUTH AFRICA, 2009 – 2013

Samantha Iyaloo[1], Karen Keddy[2], Nicola Page[2], Bhavani Poonsamy[1],Cheryl Cohen[3] and John Frean[1]

[1]Centre for Opportunistic, Tropical and Hospital Infections, NICD
[2]Centre for Enteric Diseases, NICD
[3]Centre for Respiratory Diseases and Meningitis, NICD

Introduction

Diarrhoeal disease is a major cause of morbidity and mortality in children less than 5 years old, particularly in Africa, where it is estimated to cause 12% of all childhood deaths.[1-3] In South African children between the ages of one month and one year, mortality rates in the poorest quintile are four times higher than in the wealthiest quintile. The majority of these deaths (82.6%) are caused by five conditions one of which is diarrhoeal disease, accounting for 20.7% of these deaths.[4]

While the major cause of childhood diarrhoea is rotavirus, the implementation and roll out of the rotavirus vaccination programme in 2009 has seen a significant decrease in the incidence of severe rotavirus gastroenteritis requiring hospitalisation in children less than one year.[5] There is therefore renewed interest in diarrhoeal diseases due to non-viral pathogens. Diarrhoeagenic *Escherichia coli, Salmonella, Shigella* and *Campylobacter* species are the most common bacterial pathogens[3,6,] while *Cryptosporidium* species and *Giardia lamblia* are the most common parasitic diarrhoeal pathogens in children less than 5 years.[7]

The findings from the diarrhoeal sentinel surveillance programme in South Africa, based on bacterial and parasitic isolates, are reported for the period April 2009 to December 2013. The data for 2014 are yet to be verified and will be presented in subsequent reports.

Methods

In 2009, national surveillance for rotavirus and other diarrhoeal pathogens in children less than 5 years old was set up by the National Institute for Communicable Diseases (NICD). Five sentinel sites were established in three of South Africa's provinces namely: Gauteng (Dr George Mukhari and Chris Hani Baragwanath Hospitals), Mpumalanga (Mapulaneng and Matikwana Hospitals) and KwaZulu-Natal (Edendale Hospital). Children less than 5 years of age, who were admitted to a sentinel site with symptoms of three or more loose stools in the past 24 hours, were enrolled into the programme after informed consent was obtained. A stool specimen was collected from each patient and subsequently tested for rotavirus and other enteric viruses at the Centre for Enteric Diseases (CED) Virology Laboratory at the NICD. If a stool sample was not available, nappy liners and/or rectal aspirates were collected. If a sufficient quantity of sample was left over, additional tests were conducted to identify bacterial and parasitic enteric pathogens. These additional tests were conducted at the CED Bacteriology Laboratory and the Centre for Opportunistic, Tropical and Hospital Infection's Parasitology Reference Laboratory respectively, following standard operating procedures.

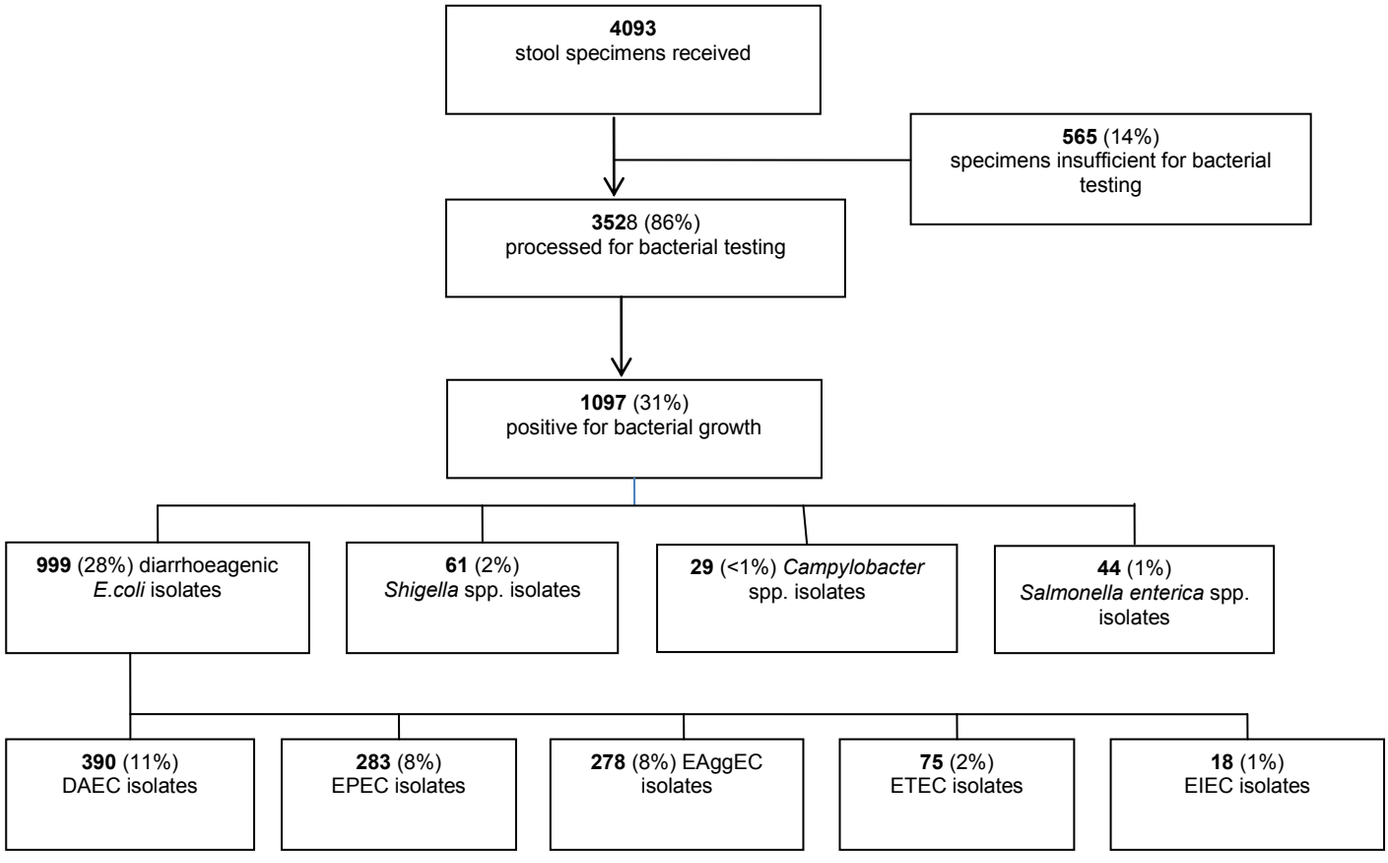
Results

Bacteria

The sentinel surveillance programme enrolled 4122 children fulfilling the case definition between April 2009 and December 2013, of which 4093 (99%) stool specimens were received by the CED Bacteriology Laboratory (figure 1). Thirty-one percent of all processed specimens were positive for bacterial growth. Twenty-

four percent (264/1097) of stool specimens tested for bacteria were co-infected with rotavirus. Co-infections between different strains of diarrhoeagenic *E.coli* (46/1097), between diarrhoeagenic *E.coli* and *Shigella* spp. (19/1097), between diarrhoeagenic *E.coli* and *Salmonella enterica* spp. (9/1097) and between diarrhoeagenic *E.coli* and *Campylobacter* (9/1097) were found amongst specimens positive for bacterial growth.

Figure 1: Flow of stool specimens received and the detection rate of bacterial pathogens amongst all specimens received during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



DAEC: Diffusely Adherent *E. coli*; EPEC: Enteropathogenic *E. coli*; EAggEC: Enteroaggregative *E. coli*; ETEC: Enterotoxigenic *E. coli*; EIEC: Enteroinvasive *E. coli*

The detection rate of pathogenic bacteria in stool was fairly constant throughout the year with a slight increase in the summer months. The detection rate of bacterial pathogens varied from 18-58% and there was an overall decrease in the detection rate after 2010 (figure 2). There was no clear seasonality in the trends by diarrhoeal pathogen although an increase in *Campylobacter* spp. isolates was observed in the latter half of 2013, following the introduction of new diagnostic protocols (figure 3).

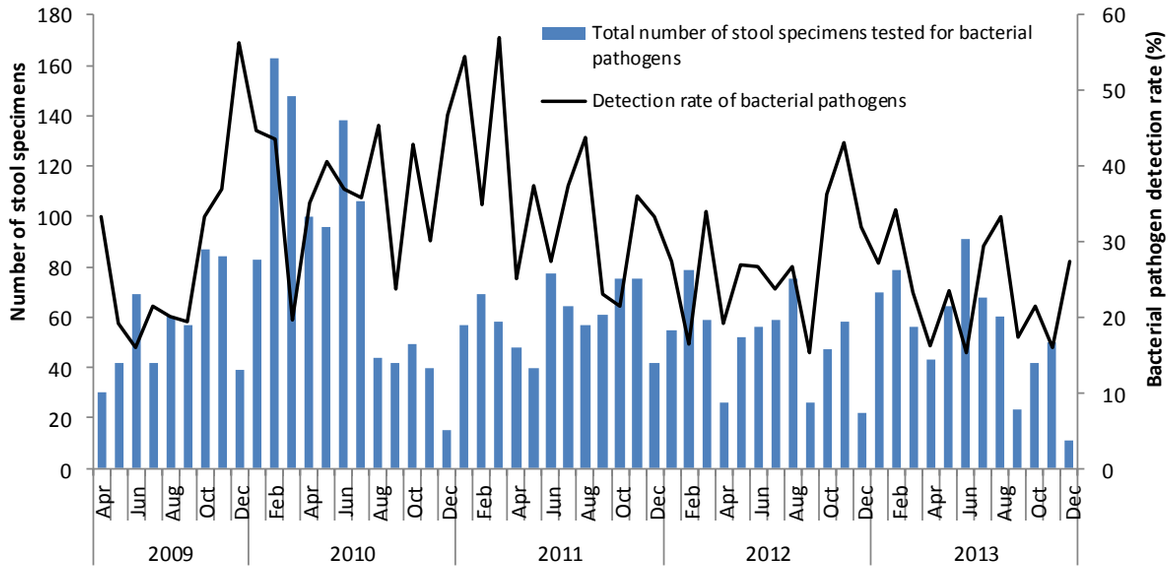
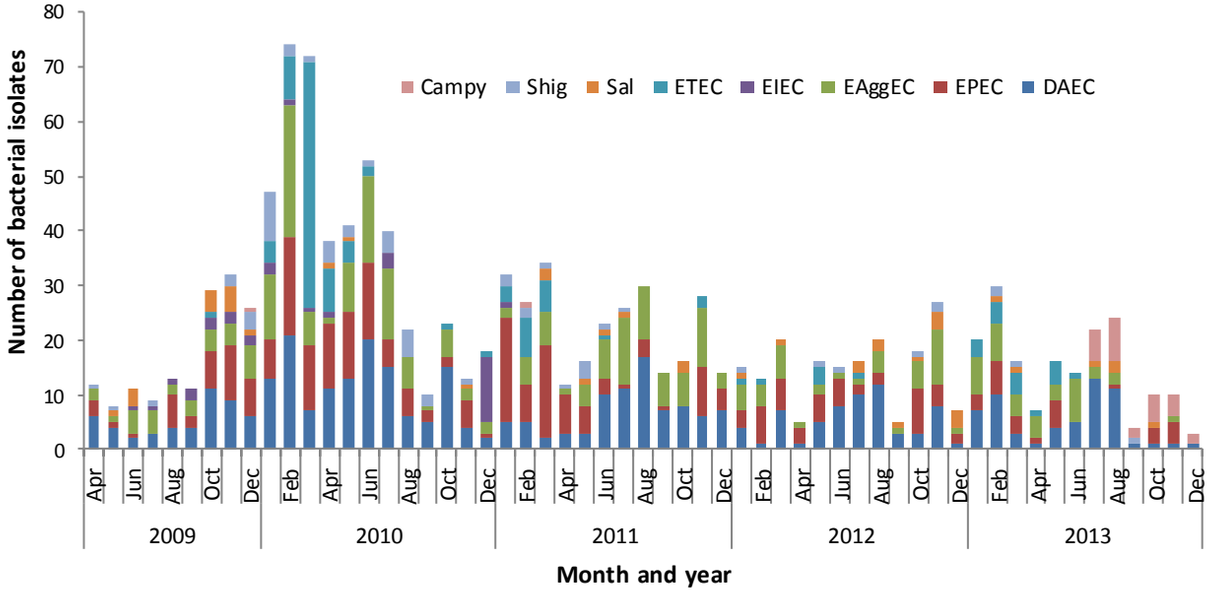


Figure 2: Numbers of stool specimens processed and the detection rate (%) of bacterial pathogens from five sentinel sites for the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Figure 3: Numbers of positive specimens by bacterial pathogen by month and year for the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



Campy= *Campylobacter* spp., Shig= *Shigella* spp., Sal= *Salmonella enterica* spp., ETEC= enterotoxigenic *E. coli*, EIE=: enteroinvasive *E.coli*, EPEC= enteropathogenic *E. coli* and DAEC= diffusely adherent *E. coli*

The highest detection rate of bacterial cases was in the 6-11 months age group (30%) followed by the <6 months age group (table 1).The lowest detection rate was amongst the 36-59 months age group (4%). The largest proportion of isolates was received from Chris Hani Baragwanath Hospital which also had the highest detection rate of all the bacterial pathogens compared to the other hospitals. There was a lower detection rate of

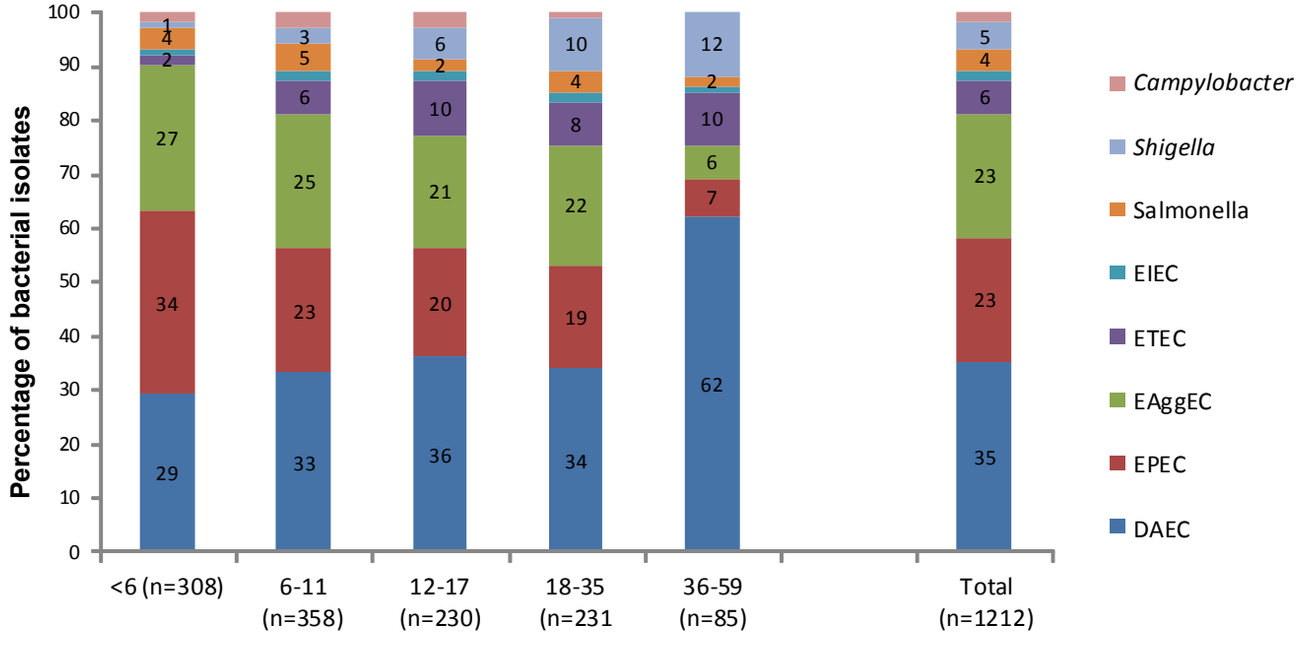
bacterial pathogens in females compared to males.

A large proportion of diarrhoeal cases were due to diffusely adherent *E. coli* (DAEC), followed by enteropathogenic *E.coli* (EPEC) and enteroaggregative *E.coli* (EAggEC) (figure 4). The largest proportion of DAEC occurred in the 36-59 months age group, whereas the proportion of EPEC, EAggEC and *Campylobacter* spp. decreased with increasing age. Enterotoxigenic *E.coli* (ETEC) and *Shigella* proportions increased with increasing age.

Table 1: Proportions of bacterial pathogens (including total) by age, gender and sentinel site in children less than 5 years admitted with diarrhoea, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Variable	Total bacterial detection n/N (%)	Diarrhoeagenic *E. coli* n/N (%)	*Shigella* spp. n/N (%)	*Salmonella enterica* spp. n/N (%)	*Campylobacter* spp. n/N (%)
Age group (months)					
<6	293/1097 (27)	270/999 (27)	5/61 (8)	12/44 (27)	7/29 (24)
6-11	331/1097 (30)	304/999 (30)	11/61 (18)	17/44 (39)	12/29 (43)
12-17	213/1097 (19)	194/999 (19)	14/61 (23)	4/44 (9)	7/29 (24)
18-35	212/1097 (19)	193/999 (19)	21/61 (34)	9/44 (20)	3/29 (10)
36-59	48/1097 (4)	38/999 (4)	10/61 (16)	2/44 (<1)	0/29 (0)
Gender					
Female	480/1097 (44)	480/999 (48)	28/61 (46)	18/44 (41)	13/29 (45)
Site					
Chris Hani Baragwanath	478/1097 (44)	425/999 (43)	37/61 (61)	18/44 (41)	17/29 (59)
Mapulaneng	99/1097 (9)	90/999 (9)	3/61 (5)	4/44 (9)	4/29 (14)
Matikwane	244/1097 (22)	230/999 (23)	6/61 (10)	7/44 (16)	5/29 (17)
Dr George Mukhari	214/1097 (20)	200/999 (20)	11/61 (18)	13/44 (30)	0/29 (0)
Edendale	62/1097 (6)	54/999 (5)	4/61 (7)	2/44 (5)	3/29 (10)

Figure 4: Proportions of bacterial pathogens and strains isolated by age group during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



EIEC = enteroinvasive *E.coli*, ETEC = enterotoxigenic *E.coli*, EAggEC = enteroaggregative *E.coli*,

Parasites

Of the 4122 children enrolled into the sentinel surveillance programme, a total of 2225 (54%) stool specimens was processed for parasites. The detection rate of parasites was 12% (271) of which 266/271 (98%) were positive for *Cryptosporidium* spp. (figure 5).

Of 2072 specimens tested for both bacteria and parasites, 81 (4%) had co-infections where both bacteria and parasites were isolated. The co-infection rate between rotavirus and parasites was 6% (17/271) amongst specimens which were positive for parasites.

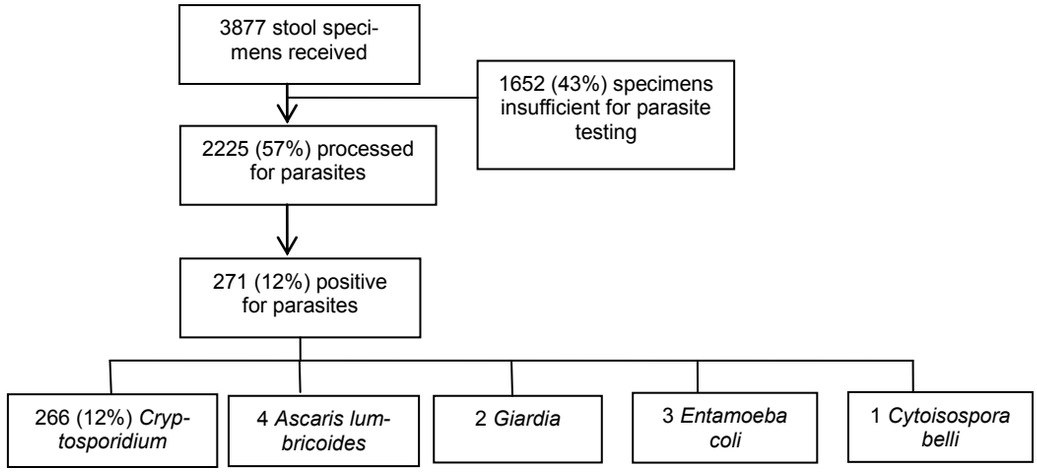


Figure 5: Flow of samples received and the numbers of parasites detected during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

There was a peak in the detection rate of parasites towards the end of the summer months (figure 6) and an overall decrease in the number of stool specimens tested for parasites in the 2011-2013 period compared to 2010. *Cryptosporidium* spp. were most common in the older age groups (figure 7). The highest detection rate of *Cryptosporidium* spp. was at Chris Hani Baragwanath Hospital (14%) and most cases of *Cryptosporidium* spp. were seen in males (table 2).

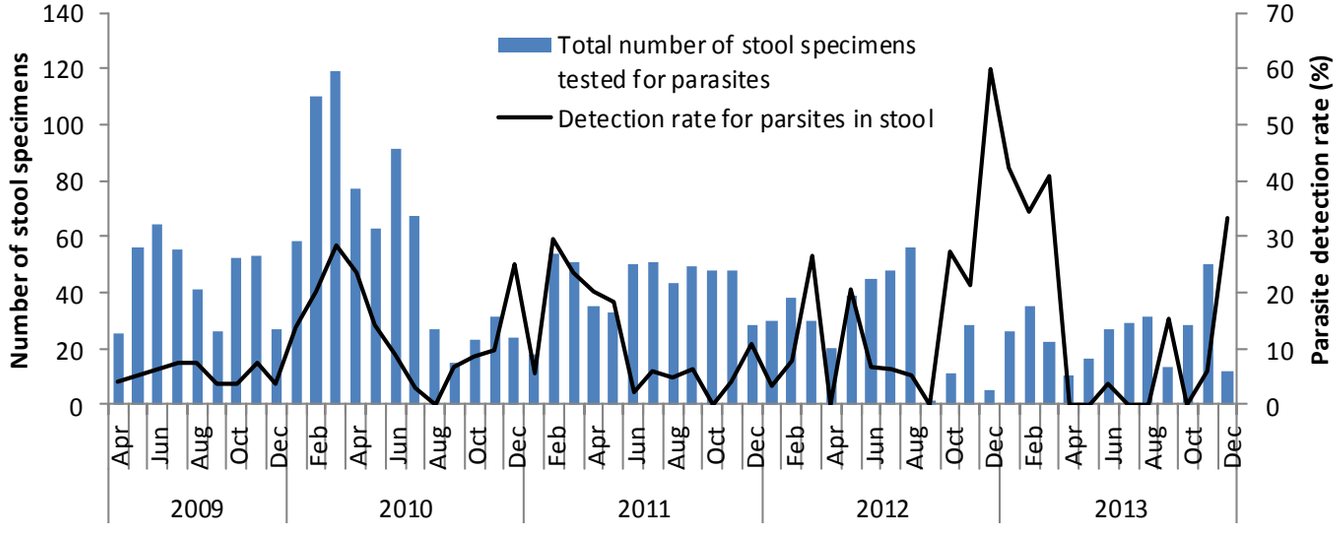


Figure 6: Numbers of stool specimens and the detection rates of parasites by month and year, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

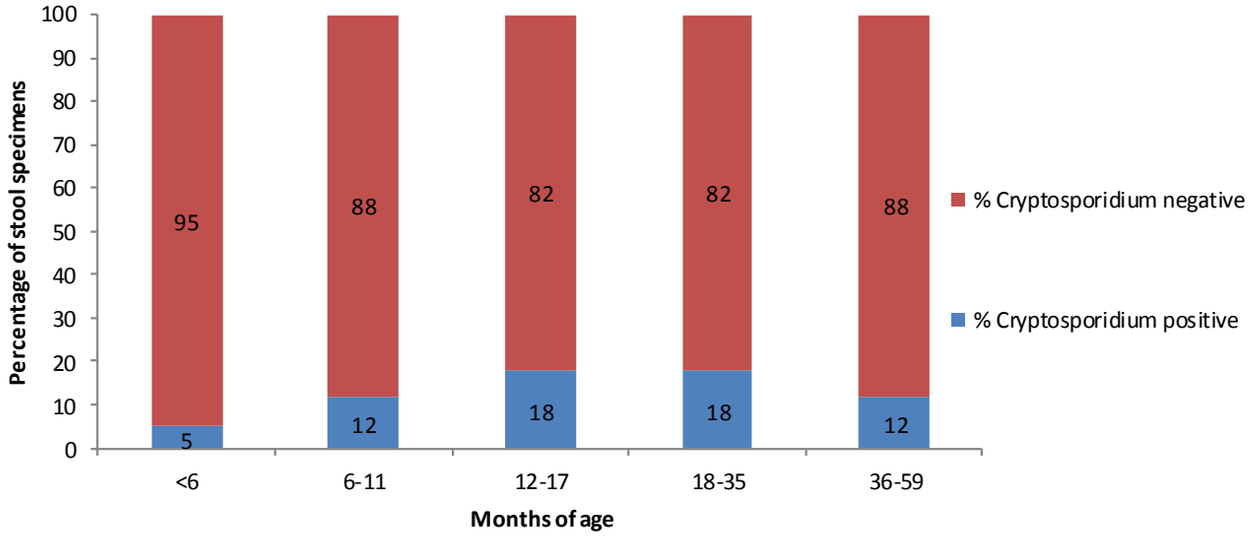


Figure 7: Detection rate of *Cryptosporidium* spp. by age (months), April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Table 2: Numbers of stool specimens collected and detection rate (%) by age, gender and sentinel site in children less than 5 years admitted with diarrhoea where *Cryptosporidium* spp. was detected, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Variable	Total specimens	Number of *Cryptosporidium* spp. positive specimens	Detection rate of *Cryptosporidium* spp. (%)
Age group (months)			
<6	634	30	5
6-11	779	91	12
12-17	415	75	18
18-35	343	60	17
36-59	91	10	11
Gender			
Male	1270	165	13
Female	954	101	11
Site			
Chris Hani Baragwanath	800	115	14
Mapulaneng	183	13	7
Matikwane	378	40	11
Dr George Mukhari	740	82	11
Edendale	124	13	10

Discussion

The rotavirus and other diarrhoeal pathogens surveillance programme enrols moderate to severe cases of diarrhoea which require hospitalisation and therefore does not represent the true burden and causes of diarrhoeal disease in children less than 5 years. The decrease in bacterial and parasitic isolates detected after 2010 may be due to a combination of interventions such as the prevention of mother to child transmission (PMTCT) of HIV, the implementation of the

rotavirus vaccine and other hygiene initiatives. Diarrhoeagenic *E.coli* was the most commonly detected pathogen. There appeared to be increases in bacterial and parasitic cases during the summer months. This most likely coincides with water-related recreational activities, rainfall patterns and increased ambient temperatures (which aid the growth of bacteria) during the summer period. ETEC diarrhoeal infections have previously been shown to decrease in older children with the bulk of cases occurring in children <2 years, whereas the data presented here show that ETEC incidence increases in age groups older than 2 years.[8]

Genotyping of a subset of the isolates obtained during this surveillance programme showed that the *Cryptosporidium* spp. infections were mostly *C. hominis* and anthroponotic *C. parvum*, indicating that humans, rather than animals, were the major sources of infection.[9]

Chris Hani Baragwanath Hospital (CHBH) had the largest proportion of pathogenic bacteria and parasites isolated from stool, which may suggest case clustering. The close proximity of CHBH to NICD may result in faster specimen processing times and better bacterial isolation rates, which may be another reason for the increased proportion of bacterial cases seen.

A major limitation of this analysis is the occurrence of co -infections involving multiple viral, bacterial and parasitic diarrhoeal pathogens and discriminating between the likely pathogenic causative agents of the diarrhoea and non-pathogenic intestinal carriage. In addition, being a hospital-based study, children under five years with severe diarrhoea presenting to clinics or private practitioners would not be identified, precluding the generalisability of these results to less severe diarrhoeal disease. Further in-depth epidemiological analysis will need to be undertaken to address these issues.

This is the first report of non-viral stool pathogens from this diarrhoeal surveillance programme. Improvements in the surveillance programme have since been made to increase the number of healthcare facilities participating in the diarrhoeal surveillance and the incorporation of point-of-care diagnostic tests to bring the surveillance system closer to real-time is being planned. This will allow for prompt investigation of clusters and possible outbreaks so that enhanced interventions and programmes in affected communities can be implemented.

Acknowledgements

The following are thanked and acknowledged for their contribution to rotavirus and other diarrhoeal pathogens surveillance programme:

- Staff from the Centre for Enteric Diseases, NICD
- Staff from the Centre for Opportunistic, Tropical and Hospital Infections, NICD
- Surveillance Officers and the data entry team
- Meera Chhagan, Michelle Groome, Kathleen Kahn, Shabir Madhi, Jocelyn Moyes, Jeff Mphahlele, Mapaseka Seheri, Sibongile Walaza
- GlaxoSmithKline for funding of the Rotavirus Surveillance Programme
- All patients and parents/guardians who have participated in this programme

References

1. Liu L, Johnson H, Cousens S, Peren J, Scott S, Lawn J, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; 379: 2151–61.
2. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries *Bull World Health Organ* 2008; 86: 710–17.
3. Lanata C, Fischer-Walker C, Olascoaga A, Torres C, Aryee M, Black R. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. *PLoS One* 2013; 8(9): e72788.
4. Day C, Gray A, Budgell E. Health and related indicators. In: Padarath A, English R, editors. South African Health Review 2012/13. Durban: Health Systems Trust; 2013. p. 119-248
5. Madhi S, Cunliffe N, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhoea in African infants. *N Engl J Med* 2010; 362: 289–98.
6. UNICEF/WHO, Diarrhea: Why children are still dying and what can be done, The United Nations Children's Fund / World Health Organization report, 2009;1-60.
7. Vargas M, Gascon J, Casals C, Schellenberg D, Urassa H, et al. (2004) Etiology of diarrhea in children less than five years of age in Ifakara, Tanzania. *Am J Trop Med Hyg* 2004:70; 536-539.
8. Walker C, Black R. Diarrhoea morbidity and mortality in older children, adolescents, and adults. *Epidemiol Infect* 2010; 138(9): 1215–26.
9. Abu Samra N, Thompson PN, Jori F, Frean J, Poonsamy B, du Plessis D, Mogoye B, Xiao L. Genetic characterization of *Cryptosporidium* spp. in diarrhoeic children from four provinces in South Africa. *Zoonoses and Public Health* 2012; 60(2):154-9.